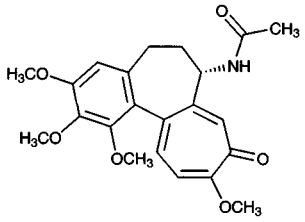
Colchicine



Molecular formula: $C_{22}H_{25}NO_6$
Molecular weight: 399.44
CAS Registry No.: 64-86-8
Merck Index: 2536
Lednicer No.: 1 152

SAMPLE

Matrix: bile, blood, gastric contents, tissue, vitreous humor
Sample preparation: Homogenize 10 g freshly minced liver with 10 mL water, adjust pH to 10 with 1 M NaOH, add 10 mg subtilisin (Sigma), heat at 55° for 1 h, adjust pH to 7.0 ± 0.5 with dilute mineral acid. 1 mL Liver homogenate, whole blood, plasma, bile, vitreous humor, or stomach contents + 1 mL 1 M $NaHCO_3$ + 7 mL dichloromethane, agitate gently for 30 min, centrifuge at 3500 rpm for 10 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 µL 0.2% phosphoric acid, inject a 50 µL aliquot.

HPLC VARIABLES

Guard column: Novapak C18
Column: 150 × 4.6 5 µm Novapak C18
Mobile phase: MeCN:MeOH:buffer 13.4:26.6:60 (Buffer was 100 mM pH 6.0 KH_2PO_4 containing 5 µM pentanesulfonic acid.)
Flow rate: 0.8
Injection volume: 50
Detector: UV 245

CHROMATOGRAM

Retention time: 6.6
Limit of detection: 5 ng/mL

KEY WORDS

plasma; whole blood; liver

REFERENCE

McIntyre,I.M.; Ruszkiewicz,A.R.; Crump,K.; Drummer,O.H. Death following colchicine poisoning, *J.Forensic Sci.*, **1994**, *39*, 280–286.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50

Detector: UV 244

CHROMATOGRAM

Retention time: 3.07
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: 4.0 mL Blood, plasma, or urine + 20 μL 1.0 μg/mL tofisopam in MeOH + 1.5 mL pH 8.0 dibasic ammonium phosphate + 4.5 mL dichloromethane, gently shake horizontally for 10 min, centrifuge at 3500 g for 10 min. Transfer the lower organic layer to 5 mL tube and evaporate under reduced pressure at 45° to 1.0 mL. Transfer into 1.5 mL Eppendorff-type plastic microtube and evaporate to dryness. Add 30 μL mobile phase, vortex for 10 s, centrifuge at 10 000 g for 5 min, inject a 0.6 μL aliquot of the

supernatant. (Equilibrate the column at least 3 h before analyzing. At the end of each chromatographic session clean column with MeCN:water 50:50 at 0.05 mL/min for 3 h.)

HPLC VARIABLES

Guard column: 1.0 × 0.8 5 μm C18 MGU-80 (LC Packing, Switzerland)
Column: 250 × 1.0 5 μm C18 Microbore (Alltech, USA)
Mobile phase: MeCN:2 mM pH 3.0 ammonium formate 75:25
Flow rate: 0.05
Injection volume: 0.6
Detector: MS, Perkin-Elmer Sciex API-100 double-quadrupole, OR +50 V, Q0 -10 V, IQ1 (lens) -12 V, ST (lens) -15 V, Q1 -13 V, EM +2200 V, TIC m/z 100-500 or 380-405, SIM m/z 400 ± 0.5 for colchicine, SIM m/z 383 ± 0.5 for tofisopam

CHROMATOGRAM

Retention time: 2.7
Internal standard: tofisopam (4.53)
Limit of detection: 0.6 ng/mL (SIM mode)

KEY WORDS

plasma; microbore; use PEEK tubing and injection loop

REFERENCE

Tracqui,A.; Kintz,P.; Ludes,B.; Rouge,C.; Douibi,H.; Mangin,P. High-performance liquid chromatography coupled to ion spray mass spectrometry for the determination of colchicine at ppb levels in human biofluids, *J.Chromatogr.B*, **1996**, *675*, 235–242.

SAMPLE

Matrix: blood, urine
Sample preparation: Condition a 1 mL 100 mg Bond Elut C2 SPE cartridge with one volume MeOH and two volumes of water. Dilute urine with four volumes of water. 500 μL Serum or diluted urine + 50 μL 2 μg/mL demecolcine in water, add to SPE cartridge, wash with 1 mL water, elute with three 350 μL portions of MeOH. Evaporate eluate to dryness under a stream of air at 40-50°, reconstitute in 150 μL water, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 4.6 5 μm C18
Column: 150 × 4.6 5 μm Microsorb C18
Mobile phase: MeCN:MeOH:buffer 13.4:26.6:60, adjusted to pH 6.0 with 100 mM KOH. After 10 min wash with MeCN:water 80:20 for 2 min, re-equilibrate for 12 min. (Buffer was 100 mM KH_2PO_4 containing 5 mM 1-pentanesulfonic acid.)
Flow rate: 1.5
Injection volume: 50
Detector: UV 254 (plasma) or UV 350 (urine)

CHROMATOGRAM

Retention time: 9.41
Internal standard: demecolcine (8.20)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, N-desacetylcolchicine

KEY WORDS

serum; SPE

REFERENCE

Ko,R.J.; Li,W.Y.; Koda,R.T. Determination of the antimitotic agents N-desacetylcolchicine, demecolcine and colchicine in serum and urine, *J.Chromatogr.*, **1990**, *525*, 411–418.

SAMPLE

Matrix: blood, urine

Sample preparation: Add tofisopam to whole blood, plasma, or urine, adjust pH to 8.0, extract with dichloromethane.

HPLC VARIABLES

Column: 250 × 1 5 μm C18 Microbore (Alltech)

Mobile phase: MeCN:2 mM pH 3 ammonium formate buffer 75:25

Flow rate: 0.05

Detector: MS, Perkin-Elmer Sciex API-100, ionspray +4 kV, nebulizing gas nitrogen, curtain gas nitrogen, orifice +50 V, electron multiplier +2.2 kV, SIM m/z 400

CHROMATOGRAM

Retention time: 2.70

Internal standard: tofisopam (m/z 383) (4.53)

Limit of detection: 0.6 ng/mL

KEY WORDS

whole blood; plasma; microbore

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Determination of colchicine in human biofluids by HPLC-ISP-MS, *J.Anal.Toxicol.*, **1996**, *20*, 70–70.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 244

CHROMATOGRAM

Retention time: 13.118

KEY WORDS

whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 3.3 7 μm Separon SGX
Mobile phase: 80 mM ammonium perchlorate in MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.18

OTHER SUBSTANCES
Simultaneous: amrinone, strychnine, nicotine, neostigmine, nicotine
Interfering: amitriptyline, promethazine, caffeine, procaine

REFERENCE
Eigendorf,H.G.; Nagel,S. Zur Analytik von Amrinone (Cordemcura). 2. Mitteillung: Hochdruckglüssigchromatographie [The analysis of amrinone (Cordemcura). 2. High pressure liquid chromatography], *Pharmazie*, **1987**, *42*, 631–631.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, gly-

benclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 5.09 (A), 4.15 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, di-

phenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

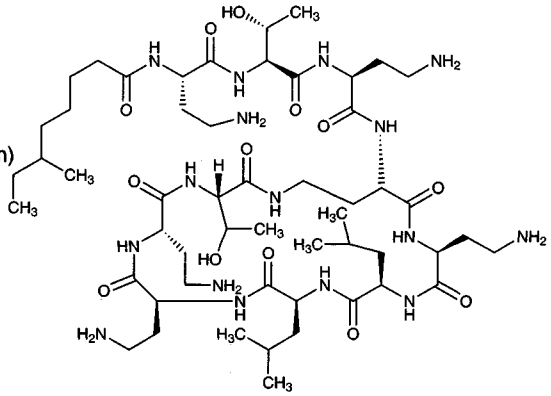
KEY WORDS

also details of plasma extraction

REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Colistin



Molecular formula: $C_{58}H_{105}N_{16}Na_5O_{28}S_5$ (colistimethate sodium)

Molecular weight: 1749.85 (colistimethate sodium)

CAS Registry No.: 1066-17-7, 1264-72-8 (colistin sulfate), 8068-28-8 (colistimethate sodium), 21362-08-3 (colistimethate sodium)

Merck Index: 2542

SAMPLE

Matrix: bulk

Sample preparation: Make up solutions in 0.1% trifluoroacetic acid, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Ultrasphere C18

Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 0:100 to 100:0 over 25 min

Flow rate: 2

Detector: UV 215

CHROMATOGRAM

Retention time: 24 (two peaks)

OTHER SUBSTANCES

Simultaneous: colistin nonapeptide

REFERENCE

Warren,H.S.; Kania,S.A.; Siber,G.R. Binding and neutralization of bacterial lipopolysaccharide by colistin nonapeptide, *Antimicrob.Agents Chemother.*, **1985**, *28*, 107–112.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 400-500 μg/mL solution in water, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Nucleosil 5 C18

Mobile phase: MeCN:buffer 22:78 (Buffer was 23 mM phosphoric acid containing 10 mM acetic acid, and 50 mM sodium sulfate, adjust pH to 2.5 with triethylamine.)

Flow rate: 0.9

Injection volume: 20

Detector: UV 220

CHROMATOGRAM

Retention time: 8 (colistin E2), 16 (colistin E1)

OTHER SUBSTANCES

Also analyzed: polymyxin B

REFERENCE

Elverdam,I.; Larsen,P.; Lund,E. Isolation and characterization of three new polymyxins in polymyxins B and E by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *218*, 653–661.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10-100 μg/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ion-pair
Mobile phase: MeCN:water:Na_3PO_4 230:700:38 (v/v/w), adjust pH to 3.0 with phosphoric acid, make up to 1000 with water
Column temperature: 27
Flow rate: 1
Injection volume: 10
Detector: UV 185 or 200

CHROMATOGRAM
Retention time: 6 (E2), 9 (E1)
Limit of detection: 30 ng (UV 185)

OTHER SUBSTANCES
Simultaneous: polymyxin

REFERENCE
Whall,T.J. High-performance liquid chromatography of polymyxin B sulfate and colistin sulfate, *J.Chromatogr.*, **1981**, *208*, 118–123.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.8-8 μm), centrifuge at 3000 rpm for 10 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS 2
Mobile phase: MeCN:water:Na_2HPO_4 230:700:15.6 (v/v/w) adjusted to pH 3.2 with phosphoric acid, make up to 1000 with water
Column temperature: 28
Flow rate: 1.4
Injection volume: 20
Detector: UV 215

CHROMATOGRAM
Retention time: 8.7 (E1 form)
Limit of detection: 3000 ng/mL

OTHER SUBSTANCES
Noninterfering: sucralfate

KEY WORDS
water

REFERENCE
Feron,B.; Adair,C.G.; Gorman,S.P.; McClurg,B. Interaction of sucralfate with antibiotics used for selective decontamination of the gastrointestinal tract, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2550–2553.

Copovithane

CAS Registry No.: 68045-74-9

SAMPLE

Matrix: blood

Sample preparation: Cool 2 mL plasma on ice for 5 min, add 200 μL 10 M perchloric acid, vortex vigorously, let stand on ice for 5 min, centrifuge at 17000 g for 15 min. Remove the supernatant and add it to 200 μL 10 M KOH, cool on ice for 5 min, centrifuge for 3 min. Remove the supernatant, add 2 mL hot (85°) saturated NaCl solution, add 3 mL chloroform, vortex vigorously, centrifuge at 17000 g for 15 min, repeat extract twice more. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 5 M HCl, heat at 160° for 16 h, cool on ice, add 1 mL 5 M NaOH, add 3 mL 1 M pH 8.0 sodium bicarbonate buffer, add 750 μL 0.5% trinitrobenzenesulfonic acid in acetone, let stand in the dark for 2.5 h, extract three times with 3 mL portions of ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 250 μL 200 mM pH 6.4 Na_2HPO_4 in MeCN, inject an aliquot. (Copovithane is hydrolyzed to methylamine and the methylamine is derivatized.)

HPLC VARIABLES

Column: 300 × 3.9 10 μm C18 (Waters)
Mobile phase: MeCN:water 30:70
Flow rate: 2
Detector: UV 340

CHROMATOGRAM

Retention time: 15
Limit of detection: 15 μg/mL

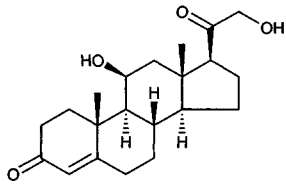
KEY WORDS

derivatization; plasma; pharmacokinetics

REFERENCE

Rosenblum,M.G.; Hortobagyi,G.N.; Wingender,W.; Hersh,E.M. Analysis of the antitumor agent Bay i 7433 (copovithane) in plasma and urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1984**, *7*, 159–166.

Corticosterone



Molecular formula: $C_{21}H_{30}O_4$
Molecular weight: 346.47
CAS Registry No.: 50-22-6
Merck Index: 2601

SAMPLE

Matrix: urine

Sample preparation: Condition a 10 mL 200 mg MCF Isolute SPE cartridge with two 3 mL portions of EtOH and two 3 mL portions of water. Centrifuge urine at 4000 g for 30 min, filter through a 0.22 μm filter unit. Dilute 0.5-1.5 mL urine to 4 mL with water. Add 40 ng IS. Add to the SPE cartridge. Wash with three 3 mL portions of water, 3 mL MeOH:10 mM NaOH 35:65, twice with 3 mL water and 3 mL MeOH:10 mM HCl 35:65. Elute with 3 mL absolute EtOH. Evaporate effluent under vacuum and reconstitute the residue with 150 μL mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES

Column: 100 × 3.2 5 μm Nucleosil 120-C18
Mobile phase: MeCN:water 26.5:73.5
Flow rate: 0.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM

Retention time: 13.01
Internal standard: 11-desoxy-17hydroxycorticosterone (15.73)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

SPE; rat

REFERENCE

Hay,M.; Mormède,P. Improved determination of urinary cortisol and cortisone, or corticosterone and 11-dehydrocorticosterone by high-performance liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *702*, 33–39.

Corticotropin

CAS Registry No.: 9002-60-2

Merck Index: 136

SAMPLE

Matrix: blood

Sample preparation: Condition a 1 mL Analytichem weak cation-exchange (carboxymethylhydrogen form, CBA) SPE cartridge with 1 mL 1% trifluoroacetic acid in MeOH, 1 mL MeOH, and 2 mL water. Add 1 mL plasma to the SPE cartridge, rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with 1 mL 1% trifluoroacetic acid in water, wash with 2 mL water, wash with 2 mL MeOH, elute with 2 mL 1% trifluoroacetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:buffer 50:50, inject a 5-75 μL aliquot. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.) [Procedure was not necessarily validated for this compound.]

HPLC VARIABLES

Column: 250 × 2 5 μm Ultrasphere octyl

Mobile phase: Gradient. A was MeOH containing 10 mM sodium octanesulfonate. B was buffer containing 10 mM sodium octanesulfonate. A:B from 45:55 to 70:30 over 30 min, maintain at 70:30 for 1 h. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.)

Column temperature: 60

Flow rate: 0.3

Injection volume: 5-75

Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with 400 mM NaOH pumped at 0.15 mL/min and 0.05% ninhydrin pumped at 0.05 mL/min and the mixture flowed through a 12 m × 0.33 mm i.d. reaction coil at 70° to the detector.

CHROMATOGRAM

Retention time: 45

Limit of detection: 100 fmole

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin II, angiotensin III, atrial natriuretic peptide, bombesin, bradykinin, gonadorelin (LHRH), somatoliberin, vasopressin

KEY WORDS

plasma; SPE; post-column reaction

REFERENCE

Rhodes,G.R.; Boppana,V.K. High-performance liquid chromatographic analysis of arginine-containing peptides in biological fluids by means of a selective post-column reaction with fluorescence detection, *J.Chromatogr.*, **1988**, *444*, 123–131.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: Gradient. A was 0.08% trifluoroacetic acid. B was MeCN:0.08% trifluoroacetic acid 70:30. A:B from 70:30 to 50:50 over 30 min.

Flow rate: 1

Detector: UV 206

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: adrenocorticotropic hormone fragments, melanotropin

KEY WORDS
human

REFERENCE
McDermott,J.R.; Smith,A.I.; Biggins,J.A.; Al-Noaemi,M.C.; Edwardson,J.A. Characterization and determination of neuropeptides by high-performance liquid chromatography and radioimmunoassay, *J.Chromatogr.*, **1981**, *222*, 371–379.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in 100 mM NaH_2PO_4 adjusted to pH 2.1 with orthophosphoric acid, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 Aquapore RP 300 (Kontron)
Mobile phase: Gradient. A was 100 mM NaH_2PO_4 adjusted to pH 2.1 with orthophosphoric acid. B was MeOH. A:B from 90:10 to 35:65 over 180 min.
Flow rate: 1
Injection volume: 100
Detector: UV 225

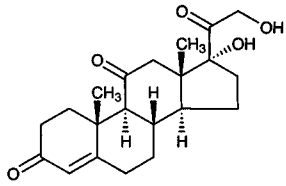
CHROMATOGRAM
Retention time: 145

OTHER SUBSTANCES
Simultaneous: adrenocorticotropin hormone fragments, lipotropic hormone and fragments, melanotropin, endorphins, prolactin, somatropin, menotropins

KEY WORDS
pig

REFERENCE
Richter,W.O.; Schwandt,P. Separation of neuropeptides by HPLC: evaluation of different supports, with analytical and preparative applications to human and porcine neurophysins, β-lipotropin, adrenocorticotropic hormone, and β-endorphin, *J.Neurochem.*, **1985**, *44*, 1697–1703.

Cortisone



Molecular formula: $C_{21}H_{28}O_5$
Molecular weight: 360.45
CAS Registry No.: 53-06-5, 50-04-4 (acetate), 509-00-2 (21β-cyclopentanepropionate), 508-95-2 (phosphate)
Merck Index: 2602
Lednicer No.: 1 188, 190

SAMPLE

Matrix: amniotic fluid, blood
Sample preparation: Centrifuge serum or amniotic fluid for 10 min. 0.5-1 mL Serum or amniotic fluid + 500 μL MeOH:water 5:95, mix, inject 750 μL onto column A with mobile phase A, after 5 min elute contents of column A onto column B with mobile phase B, monitor effluent from column B.

HPLC VARIABLES

Column: A Serumont-25 (Sekisui); B 260 × 4.6 5 μm Medipola-ODS C18 (Sekisui)
Mobile phase: A water; B MeCN:MeOH:buffer 2:7:20 (Buffer was 6.8 g/L KH_2PO_4, pH adjusted to 3.1 with concentrated phosphoric acid.)
Column temperature: 40
Flow rate: A 0.8; B 1
Injection volume: 750
Detector: UV 245

CHROMATOGRAM

Retention time: 56.8
Limit of detection: 5.8 ng

OTHER SUBSTANCES

Extracted: estetrol, estriol, hydrocortisone
Noninterfering: corticosterone, testosterone, hydroxyprogesterone, androstenedione, progesterone

KEY WORDS

serum; column-switching

REFERENCE

Noma,J.; Hayashi,N.; Sekiba,K. Automated direct high-performance liquid chromatographic assay for estetrol, estriol, cortisone and cortisol in serum and amniotic fluid, *J.Chromatogr.*, **1991**, *568*, 35–44.

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge. Mix 1 mL plasma with 134.0 ng hydrocortisone-d_5 and 74.56 ng cortisone-d_5. Add the sample to the SPE cartridge, wash with 8 mL water, elute with 4 mL ethyl acetate, evaporate the eluate to dryness at 70° under a stream of nitrogen, dissolve the residue in 30 μL mobile phase, filter (0.45 μm), inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.0 4 μm LiChroCART Superspher 100
Mobile phase: A MeOH:THF:50 mM ammonium formate 17:53:180; B MeCN:50 mM ammonium formate 35:65
Flow rate: 0.6 (A); 1.3 (B)

Injection volume: 20
Detector: MS, Shimadzu LCMS-QP1000EX Model 750 B, thermospray, vaporizer control 155°, vaporizer tip 195°, vapor 274°, ion source block 295°, tip heater 305°, m/z 361

CHROMATOGRAM
Retention time: 10 (A)
Internal standard: hydrocortisone-d_5, cortisone-d_5
Limit of detection: 0.50 ng

OTHER SUBSTANCES
Extracted: hydrocortisone, prednisolone, prednisone

KEY WORDS
plasma; SPE

REFERENCE
Shibasaki,H.; Furuta,T.; Kasuya,Y. Quantification of corticosteroids in human plasma by liquid chromatography-thermospray mass spectrometry using stable isotope dilution, *J.Chromatogr.B*, **1997**, *692*, 7–14.

SAMPLE
Matrix: blood
Sample preparation: Prepare a Bond-Elut C18 SPE column by washing with 2 mL MeCN, 2 mL acetone:water 2:98, and 4 mL water. Do not allow column to run dry. 2 mL Plasma + 40 μL 5 μg/mL dexamethasone in MeOH, add to SPE cartridge, allow to sit for 15 min, wash twice with 2 mL water, wash twice with 2 mL acetone:water 2:98, pull a vacuum on the column for 15 min, elute with 1 mL MeCN under vacuum. Evaporate the eluate to dryness under a stream of nitrogen at 40°, dissolve the residue in 150 μL dichloromethane, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb Si-60
Mobile phase: Dichloromethane:water-saturated dichloromethane:THF:MeOH:glacial acetic acid 664.5:300:10:25:0.5
Flow rate: 0.8
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 14
Internal standard: dexamethasone (23.5)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: prednisolone acetate, prednisone, hydrocortisone, prednisolone

KEY WORDS
plasma; normal phase; pig; SPE

REFERENCE
Prasad,V.K.; Ho,B.; Haneke,C. Simultaneous determination of prednisolone acetate, prednisolone, prednisone, cortisone and hydrocortisone in swine plasma using solid-phase and liquid-liquid extraction techniques, *J.Chromatogr.*, **1986**, *378*, 305–316.

SAMPLE
Matrix: blood

Sample preparation: Condition a Tef Elutor C18 cartridge with two 3 mL portions of MeOH then two 3 mL portions of water. 1 mL Plasma + 50 μL 400 ng/mL flumethasone in 5:95 MeOH:water, heat at 50° for 10 min, add to cartridge, wash with 2 mL water, 1 mL MeOH:water 10:90, 4 mL acetone:water 20:80, apply suction to cartridge for 10 min to air dry. Elute with 1 mL MeOH, evaporate eluent at 45° under nitrogen, reconstitute with 50 μL mobile phase, inject 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 2 3 μm C18 Hypersil
Mobile phase: MeCN:THF:water 8:10:82, containing 5 mL/L triethylamine, pH adjusted to 6.5 with citric acid
Flow rate: 0.6
Injection volume: 25
Detector: UV 242

CHROMATOGRAM

Retention time: 2.90
Internal standard: flumethasone (11.50)
Limit of detection: 300 pg/mL

OTHER SUBSTANCES

Simultaneous: prednisone, hydrocortisone, adrenosterone, prednisolone, estriol, corticosterone, methylprednisolone, dexamethasone, hydroxyprogesterone, testosterone, deoxycorticosterone, fluorometholone, spironolactone, equilenin, estrone, estradiol, progesterone, diphenhydramine, propranolol, aspirin, theophylline, imipramine, desipramine, indomethacin, amitriptyline, nortriptyline, nordiazepam, diazepam, chlordiazepoxide
Noninterfering: caffeine, nicotine, cotinine, chlorothiazide, acetazolamide, phenytoin, pheniramine, cephalothin, primidone, acebutolol, hydrochlorothiazide, quinine, acetophenetidine, furosemide, aldosterone, triamcinolone, ephedrine, allopurinol, phenylephrine
Interfering: tripelennamine, carbamazepine, probenecid, phenobarbital

KEY WORDS

plasma; SPE

REFERENCE

Hariharan,M.; Naga,S.; VanNoord,T.; Kindt,E.K. Simultaneous assay of corticosterone and cortisol in plasma by reversed-phase liquid chromatography, *Clin.Chem.*, **1992**, *38*, 346–352.

SAMPLE

Matrix: blood
Sample preparation: Condition a 2 mL 200 mg Tef Elutor C18 SPE cartridge (Versa Prep) with 3 mL MeOH and two 3 mL portions of water. 1 mL Plasma + 50 μL 400 ng/mL flumethasone in MeOH:water 5:95, heat at 50° for 10 min, add to SPE cartridge, wash with 2 mL water, wash with 1 mL MeOH:water 10:90, wash with 4 mL acetone:water 20:80, air-dry for 10 min, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 50 μL mobile phase, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 2 3 μm Hypersil
Mobile phase: MeCN:THF:water 8:10:82 containing 5 mL/L triethylamine, pH adjusted to 6.5 with citric acid
Flow rate: 0.6
Injection volume: 25
Detector: UV 242

CHROMATOGRAM

Retention time: 4

Internal standard: flumethasone (13)
Limit of detection: 0.3 ng/mL

OTHER SUBSTANCES

Extracted: hydrocortisone, corticosterone
Simultaneous: acebutolol, acetazolamide, acetophenetidin, adrenosterone, aldosterone, amitriptyline, androsten-3,17-dione, aspirin, cephalothin, chlordiazepoxide, chlorothiazide, dehydrocorticosterone, deoxycorticosterone, deoxycortisol, desipramine, dexamethasone, diazepam, diphenhydramine, equilenin, estradiol, estriol, estrone, fluorometholone, furosemide, hydrochlorothiazide, hydroxycorticosterone, hydroxyprogesterone, hydroxyprogesterone, imipramine, indomethacin, methylhydroxyprogesterone, methylprednisolone, nandrolone, nordiazepam, nortriptyline, pheniramine, phenobarbital, phenytoin, prednisolone, prednisone, primidone, probenecid, progesterone, propranolol, quinine, spironolactone, testosterone, theophylline, triamcinolone
Noninterfering: caffeine, nicotine, cotinine, ephedrine, allopurinol, phenylephrine
Interfering: tripelennamine, carbamazepine

KEY WORDS

serum; SPE

REFERENCE

Hariharan,M.; Naga,S.; VanNoord,T.; Kindt,E.K. Assay of human plasma cortisone by liquid chromatography: normal plasma concentrations (between 8 and 10 a.m.) of cortisone and corticosterone, *J.Chromatogr.*, **1993**, *613*, 195–201.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 10 μL IS in water, extract twice by shaking for 1 min with 1.2 mL dichloromethane, evaporate organic layer below 40° under reduced pressure, dissolve residue in 100 μL MeCN. Add 10 μL reagent 1, add 10 μL reagent 2, heat at 70° for 20 min, cool to room temperature, add 100 μL water, add 200 μL MeOH:water 1:1, add to Sep-Pak C18 cartridge, wash vial with 2 mL MeOH:water 1:1 and add washings to cartridge, wash cartridge with 40 mL MeOH:water 1:1, elute with 5 mL MeOH. Concentrate eluent to 500 μL by evaporation at 40° under reduced pressure, inject 20 μL aliquot. (Reagent 1 was 30 mg 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole in 3 mL pyridine, add 700 mg 4-piperidinopyridine, dilute to 10 mL with MeCN. Reagent 2 was 700 mg 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate in 10 mL MeCN. Prepare 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole as follows. Add 13 g 4-carboxybenzaldehyde (terephthalaldehydic acid) in 400 mL EtOH dropwise to 4,5-dimethyl-1,2-phenylenediamine in 400 mL EtOH in an ice bath, after 1 h reflux for 8 h, cool to room temperature, collect the precipitate, recrystallize three times from MeOH:water 50:50 to give 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole as a white amorphous product (mp >300°) (J.Chromatogr. 1991, 585, 219). 4-Piperidinopyridine is not commercially available but 4-dimethylaminopyridine or 4-pyrrolidinopyridine can be used instead although interferences are greater (J. Chromatogr. 1991, 585, 219). Alternatively 4-piperidinopyridine can be synthesized as follows. Add 200 mmoles piperidine dropwise with stirring to 15 g phosphorus pentoxide and 9.51 g 4-hydroxypyridine, heat at 250° for 7 h, cautiously pour onto 200 g ice, add 400 mL 1 M NaOH, add 200 mL ether. Remove the ether layer and extract the aqueous layer three times with 100 mL portions of ether. Combine the organic layers and dry them over anhydrous potassium carbonate, evaporate, distil the residue, recrystallize from petroleum ether (bp 80-100°) to give 4-piperidinopyridine (bp 167-170°/11 mm Hg; mp 79-80°) (Synthesis 1978, 844). Alternatively, add 1.94 g 4-bromopyridine hydrochloride to 5 mL 50% NaOH, add 5 mL piperidine, add 2.72 g benzyltriethylammonium bromide, heat at 100° for 5 h, remove excess piperidine by distillation, add 25 mL water, extract four times with 25 mL portions of benzene. Combine the organic layers and dry them over anhydrous sodium sulfate, boil the residue with petroleum ether to give 4-piperidinopyridine (mp 80°) (Syn. Commun. 1979, 9, 251). Prepare 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate as follows. Stir 1.41 moles isopropy-

lisocyanate in 750 mL dichloromethane at 5°, add 144 g 3-dimethylaminopropylamine (N,N-dimethyl-1,3-propanediamine) in 250 mL dichloromethane at such a rate that the temperature does not exceed 10°, add 500 mL triethylamine, add 300 g p-toluenesulfonyl chloride in 300 mL dichloromethane at such a rate that the temperature does not exceed 10°, reflux for 3 h, add 400 g anhydrous sodium carbonate, add 3.5 L ice water, stir vigorously for 30 min, remove the organic phase. Extract the aqueous phase three times with 500 mL portions of dichloromethane. Combine the organic layers and dry them over anhydrous sodium sulfate, evaporate under reduced pressure, distil the residue to give 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide (bp 91-92°/10 mm Hg (Ber. 1941, 74B, 1285)) (cf. Org. Syn. 1973, Coll. Vol. V, 555). Prepare pyridine perchlorate from pyridine and 20% perchloric acid, crystallize from EtOH (Ber. 1926, 59, 446). Add 18 g pyridine perchlorate in portions to 100 mmoles 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide stirred in 200 mL dichloromethane at 0°, let stand for 30 min, filter, add 200 mL anhydrous diethyl ether to the filtrate. Filter off the precipitate and recrystallize it from dichloromethane/diethyl ether to give 1-isopropyl-3-(3-dimethylaminopropyl)carbodiimide perchlorate (mp 88-90°) (Chem. Pharm. Bull. 1985, 33, 5375).)

HPLC VARIABLES

Guard column: 50 × 4.6 7 μm Zorbax ODS
Column: 250 × 4.6 7 μm Zorbax ODS
Mobile phase: MeOH:water 75:25 containing 5 mM tetramethylammonium hydrogen sulfate
Flow rate: 0.4
Injection volume: 20
Detector: F ex 334 em 418

CHROMATOGRAM

Retention time: 25.2
Internal standard: fluocinolone acetonide (40.7)
Limit of detection: 0.6-3 pg/mL

OTHER SUBSTANCES

Simultaneous: aldosterone, corticosterone, hydrocortisone, dexamethasone, triamcinolone

KEY WORDS

plasma; derivatization

REFERENCE

Katayama,M.; Masuda,Y.; Taniguchi,H. Determination of corticosteroids in plasma by high-performance liquid chromatography after pre-column derivatization with 2-(4-carboxyphenyl)-5,6-dimethylbenzimidazole, *J.Chromatogr.*, **1993**, *612*, 33–39.

SAMPLE

Matrix: blood
Sample preparation: Prepare a Sep-Pak Plus Environmental C18 cartridge by washing with 15 mL MeOH then 15 mL water. 1 mL Serum + 100 μL 3 μg/mL betamethasone in isopropanol:MeCN 1:1 + 100 μL isopropanol:acetonitrile 1:1, mix, add to SPE cartridge, wash with 10 mL water, elute with 3 mL MeOH. Evaporate the eluate at 50° under a stream of nitrogen, reconstitute in 200 μL mobile phase A, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: μBondapak C18 guard column
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. A was isopropanol:50 mM pH 4.5 acetate buffer 10:90. B was isopropanol:50 mM pH 4.5 acetate buffer 30:70. A:B from 90:10 to 30:70 over 25 min, hold at 30:70 for 5 min, to 90:10 over 5 min, hold at 90:10 for 15 min before next injection.
Column temperature: 40
Flow rate: 1

Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 24
Internal standard: betamethasone (33)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites, prednisone, hydrocortisone, prednisolone

KEY WORDS
serum; SPE

REFERENCE
Hirata,H.; Kasama,T.; Sawai,Y.; Fike,R.R. Simultaneous determination of deflazacort metabolites II and III, cortisol, cortisone, prednisolone and prednisone in human serum by reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *658*, 55–61.

SAMPLE
Matrix: blood
Sample preparation: Centrifuge plasma at 2500 g for 10 min, mix the supernatant with an equal volume of 1 M pH 3.0 glycine buffer containing 0.2% Tween 20, centrifuge at 2500 g for 10 min, inject an aliquot of the supernatant on to column A and elute to waste with mobile phase, after 3 min divert the effluent from column A on to column B, after 3 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Backflush column A with mobile phase for 28 min.

HPLC VARIABLES
Column: A 30 × 2.1 Spherisorb C1 pH stable; B 150 × 2.1 Spherisorb C1 pH stable
Mobile phase: 5 mM pH 7.3 Tris-nitric acid buffer containing 0.1% Tween 20 and 150 mM sodium nitrate
Column temperature: 40
Flow rate: 0.2
Injection volume: 50
Detector: UV

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Extracted: hydrocortisone, prednisolone

KEY WORDS
plasma; column-switching; heart-cut

REFERENCE
Lövgren,U.; Johansson,M.; Kronkvist,K.; Edholm,L.-E. Biocompatible sample pretreatment for immunochemical techniques using micellar liquid chromatography for separation of corticosteroids, *J.Chromatogr.B*, **1995**, *672*, 33–44.

SAMPLE
Matrix: blood
Sample preparation: Condition an Empore C8 extraction disc SPE cartridge (3M Co.) by adding 500 μL MeOH and forcing through three drops, discard the remaining liquid, add water, force through three drops, discard the water. 300 μL Serum + 150 μL IS solution, let stand at room temperature for 10 min, add 800 μL saturated sodium borate solution,

mix, centrifuge at 12400 g for 3 min (if necessary), add to SPE cartridge, centrifuge at 100-120 g for 5 min, force through 200 μL water, force through 500 μL MeOH:water 18:82, elute with 50 μL MeCN then 150 μL water, mix the eluates, inject a 20 μL aliquot. (IS solution contained 0.5 mg/L fludrocortisone and 0.75 mg/L methylprednisolone in 400 mM HCl.) (The extraction disc permits use of lower volumes of eluate than a conventional SPE cartridge.)

HPLC VARIABLES
Guard column: 20 × 2 30 μm Permaphase ETH (Du Pont)
Column: 250 × 2 Ultrasphere C18 or 250 × 4.6 Ultrasphere C18
Mobile phase: THF:water 20:80 (Use a 150 × 4.6 37-53 μm silica gel (Whatman) saturating column (held at 55°) between the pump and the injector.)
Column temperature: 55
Flow rate: 0.18 (250 × 2 column) or 0.8 (250 × 4.6 column)
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11
Internal standard: fludrocortisone (15), methylprednisolone (20)
Limit of detection: 4 ng/mL

OTHER SUBSTANCES
Extracted: hydrocortisone, prednisone, prednisolone, corticosterone
Simultaneous: aldosterone, triamcinolone, metyrapone, 11-deoxycortisol, dexamethasone, 21-deoxycortisone, androsteindione, beclomethasone, 11-deoxycorticosterone, testosterone, 17-hydroxyprogesterone, progesterone, pregnenolone

---

KEY WORDS
serum; SPE; extraction disc

REFERENCE
Lensmeyer,G.L.; Onsager,C.; Carlson,I.H.; Wiebe,D.A. Use of particle-loaded membranes to extract steroids for high-performance liquid chromatographic analyses. Improved analyte stability and detection, *J.Chromatogr.A*, **1995**, *691*, 239–246.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 100 μL water containing 5 μg/mL 2,3-diaminonaphthalene and 3.5 μg/mL 18-hydroxy-11-deoxycorticosterone + 1 mL 250 mM NaOH + 7 mL diethyl ether, shake on a rotary shaker for 15 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 70 μL MeOH:100 mM perchloric acid 50:50, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A was 58 mM NaH_2PO_4 containing 6 mM sodium heptanesulfonate, adjusted to pH 3.1 with concentrated phosphoric acid. B was MeCN:MeOH 85:15. A:B from 100:0 to 78:22 over 5 min, to 70:30 over 12 min, maintain at 70:30 for 4 min, to 65:35 over 9 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245, 256, 343

CHROMATOGRAM
Retention time: 14.73

Internal standard: 2,3-diaminonaphthalene (10.71), 18-hydroxy-11-deoxycorticosterone (15.85)
Limit of detection: 1-10 ng/mL (245 nm)

OTHER SUBSTANCES
Extracted: betamethasone, chloroquine, corticosterone, dexamethasone, fluocinolone acetonide, fluendrenolide, fluorometholone, fluprednisolone, hydrocortisone, hydroxychloroquine, 17δ-hydroxyprogesterone, meprednisone, methylprednisolone, methylprednisolone acetate, paramethasone, prednisolone, prednisone, progesterone, triamcinolone
Noninterfering: aspirin, ibuprofen, indomethacin, phenylbutazone, pregnenolone

KEY WORDS
serum

REFERENCE
Volin,P. Simple and specific reversed-phase liquid chromatographic, *J.Chromatogr.B*, **1995**, *666*, 347–353.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Urine. 1 mL Urine + 1 mL MeOH:EtOH 50:50, centrifuge at 4000 g for 10 min. Remove the supernatant and evaporate to about 200 μL under a stream of nitrogen at 37°, inject a 5-20 μL aliquot. Plasma. Mix plasma with an equal volume of MeOH:EtOH 50:50, let stand at -20° for 30 min or overnight. Remove supernatant and wash precipitate twice with equal volumes of MeOH:EtOH 50:50. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL MeOH:water 65:35, inject a 5-20 μL aliquot. Tissue. Homogenize-(Polytron) fetal tissue in 10-15 mL MeOH:dimethoxymethane 50:50 for 1 min or until breakup was complete, shake at 37° overnight, centrifuge at 4000 g for 5 min. Filter (Whatman No. 1 filter paper) supernatant. Resuspend precipitate in MeOH:dimethoxymethane 50:50, filter, wash precipitate with MeOH. Combine filtrates, evaporate to dryness under nitrogen, resuspend residue in up to 500 μL MeOH:water 65:35, centrifuge, inject a 5-20 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 70 × 6 35-50 μm Bondapak C18 Corasil
Column: 250 × 10 5 μm LiChrosorb RP-18
Mobile phase: Gradient. MeOH:10 mM pH 6.9 ammonium acetate from 10:90 to 100:0 over 50 min (Waters No. 5 convex gradient)
Flow rate: 1.5
Injection volume: 5-20
Detector: UV 254

CHROMATOGRAM
Retention time: 29.39

OTHER SUBSTANCES
Extracted: metabolites, triamcinolone, triamcinolone acetonide, hydrocortisone, cortexolone, 6β-hydroxycortisol, cortisol glucuronide

KEY WORDS
plasma; monkey

REFERENCE
Althaus,Z.R.; Rowland,J.M.; Freeman,J.P.; Slikker,W.,Jr. Separation of some natural and synthetic corticosteroids in biological fluids and tissues by high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *227*, 11–23.

SAMPLE

Matrix: blood, urine

Sample preparation: Condition a Sep-Pak Plus C18 SPE cartridge with 7 mL MeOH and 14 mL water. Add 40 ng 6β-hydroxycortisone to 400 μL plasma or urine, add the mixture to the SPE cartridge, wash with 6 mL water, 3 mL MeOH:water 12:88, and 3 mL petroleum ether, elute with 5 mL ethyl acetate. Dry the eluate under reduced pressure at 40°, add 200 μL MeCN:triethylamine 90:10 and MeCN:0.1% quinuclidine 20:80 to the residue, vortex. Add 200 μL 0.02% 9-anthroyl nitrile and a few molecular sieves (4A), let stand for 30 min, evaporate under reduced pressure at 40°, dissolve the residue in 200 μL acetone, dilute with 2 mL n-hexane. Add the mixture to a Sep-Pak Plus Silica SPE cartridge, wash with 14 mL 1,2-dichloroethane, elute with 5 mL ethyl acetate. Evaporate the eluate under reduced pressure at 40°, reconstitute the residue in 200 μL mobile phase, inject a 30-60 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Cosmosil 5SL (Nacalai Tesque, Japan)

Mobile phase: Dioxane:ethyl acetate:chloroform:n-hexane:pyridine 58.1:11.6:11.6:16.3:2.4 (Caution! Dioxane and chloroform are carcinogens!)

Flow rate: 1 for 45 min, to 1.2 over 5 min

Injection volume: 30-60

Detector: F ex 360 em 460

CHROMATOGRAM

Retention time: 28

Internal standard: 6β-hydroxycortisone (86)

Limit of detection: 100 pg/mL

OTHER SUBSTANCES

Extracted: prednisone, prednisolone, hydrocortisone, 6β-hydroxycortisol, 6β-hydroxyprednisolone

KEY WORDS

derivatization; plasma; urine; SPE; normal phase

REFERENCE

Shibata,N.; Hayakawa,T.; Takada,K.; Hoshino,N.; Minouchi,T.; Yamaji,A. Simultaneous determination of glucocorticoids in plasma or urine by high-performance liquid chromatography with precolumn fluorimetric derivatization by 9-anthroyl nitrile, *J.Chromatogr.B*, **1998**, *706*, 191–199.

SAMPLE

Matrix: formulations

Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS

Mobile phase: MeOH:water 75:25

Flow rate: 1.5

Injection volume: 10

Detector: UV 240

CHROMATOGRAM

Retention time: 3.3

Limit of detection: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine

Interfering: formebolone, benzyl alcohol, testolactone

KEY WORDS

oils; tablets; suspensions

REFERENCE

Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE

Matrix: perfusate

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH and 10 mL water. 3 mL Perfusate + 500 ng 6α-methylprednisolone, add to the SPE cartridge, wash three times with 10 mL aliquots of water, elute with 5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 Newguard RP-18
Column: two 250 × 4.6 Spheri-5 RP-18 columns in series
Mobile phase: MeOH:water 53:47
Column temperature: 40
Flow rate: 1.1
Injection volume: 50
Detector: UV 242

CHROMATOGRAM

Retention time: 16
Internal standard: 6α-methylprednisolone (30)
Limit of detection: 5 nM

OTHER SUBSTANCES

Extracted: hydrocortisone, dihydrocortisone, dihydrocortisol, metabolites
Simultaneous: prednisolone
Noninterfering: acetaminophen, albuterol, betamethasone, bupivacaine, carbamazepine, cholesterol, clonazepam, dehydroepiandrosterone, dexamethasone, diazepam, estradiol, estriol, hydroxyprogesterone, methimazole, phenobarbital, prednisone, progesterone, ritodrine, scopolamine, testosterone

KEY WORDS

SPE

REFERENCE

Dodds,H.M.; Maguire,D.J.; Mortimer,R.H.; Addison,R.S.; Cannell,G.R. High performance liquid chromatographic separation of cortisol, cortisone, and their 20-reduced metabolites in perfusion media, *J.Liq.Chromatogr.*, **1995**, *18*, 1809–1820.

SAMPLE
Matrix: solutions
Sample preparation: Prepare solutions in MeCN, dilute to an appropriate concentration with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 120 × 4.6 5 μm octadecyl Bakerbond
Mobile phase: MeCN:water 30:70 containing 16 mM β-cyclodextrin
Column temperature: 5
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 0.8

OTHER SUBSTANCES
Simultaneous: hydrocortisone, testosterone, prednisone, 17α-methyltestosterone, 17α-hydroxyprogesterone

REFERENCE
Zarzycki,P.K.; Wierzbowska,M.; Lamparczyk,H. The influence of temperature on the high performance liquid chromatographic separation of steroids using mobile phases modified with β-cyclodextrin, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1305–1311.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm SI-100 (Brownlee)
Mobile phase: Butyl chloride:THF:MeOH:glacial acetic acid 95:7:3.5:3 (Butyl chloride was 50% water saturated.)
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 12 (cortisone), 8.5 (cortisone acetate)

OTHER SUBSTANCES
Simultaneous: hydrocortisone acetate, hydrocortisone, 4-androstene-3,11,17-trione

KEY WORDS
normal phase

REFERENCE
Kane,M.P.; Tsuji,K. Radiolytic degradation scheme for ^{60}Co-irradiated corticosteroids, *J.Pharm.Sci.*, **1983**, *72*, 30–35.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38

Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 2.19 (cortisone acetate)

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS
Mobile phase: MeOH:THF:water 26:18:56
Column temperature: 48
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 5.4

OTHER SUBSTANCES
Simultaneous: estriol, cortisol, corticosterone, 11-deoxycortisol, androstenedione, prednisone acetate, 11-deoxycorticosterone, testosterone, 17α-hydroxyprogesterone, dexamethasone acetate, estradiol, estrone, progesterone

REFERENCE
Wei,J.Q.; Wei,J.L.; Zhou,X.T. Optimization of an isocratic reversed phase liquid chromatographic system for the separation of fourteen steroids using factorial design and computer simulation, *Biomed.Chromatogr.*, **1990**, *4*, 34–38.

SAMPLE
Matrix: solutions
Sample preparation: Evaporate solution (eluate from preparative HPLC) to dryness under a stream of nitrogen, reconstitute with 10 μL 2 μg/mL 9-anthroylnitrile (Wako) in MeCN and 10 μL triethylamine:MeCN 30:70 under nitrogen, let stand at room temperature for 20 min, add 5 μL water, after 6 min add 50 μL 600 mM acetic acid in MeCN, evaporate to dryness under a stream of nitrogen at 37°, reconstitute with 90 μL MeOH: 0.4 N NaH_2PO_4 60:40, add to a Cyclobond I silica-bonded β-cyclodextrin SPE cartridge (Astec), wash with 1 mL water, wash with 8 mL MeOH:water 25:75 containing 7.5 mM pH 7.0 phosphate buffer, elute with 1 mL MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with mobile phase, inject an aliquot on to column A and elute to waste with mobile phase, after the solvent front has passed through divert the effluent from column A on to column B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 30 × 2.1 silica (Brownlee); B 150 × 2 Hypersil
Mobile phase: Hexane:ethyl acetate 67:33 (half-saturated with water)
Flow rate: 0.5
Detector: F ex 305-395 em 430-470

CHROMATOGRAM
Retention time: 10.2

OTHER SUBSTANCES
Simultaneous: hydrocortisone, prednisolone

KEY WORDS
derivatization; SPE; column-switching; normal phase

REFERENCE
Haegele,A.D.; Wade,S.E. Ultrasensitive differential measurement of cortisol and cortisone in biological samples using fluorescent ester derivatives in normal phase HPLC, *J.Liq.Chromatogr.*, **1991**, *14*, 1133–1148.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN:water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 17.5

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, danazol, diflunisal, doxapram, estrone, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, testosterone, testosterone propionate, tranylcypromine, tripelennamine

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 25 μg/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10 ODS-1

Mobile phase: MeOH:water 55:45
Column temperature: 40
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM
Retention time: k' 0.9437

OTHER SUBSTANCES
Also analyzed: androsterone (UV 210), cortexolone (UV 240), estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), hydroxyprogesterone (UV 240), lynestrenol (UV 210), medroxyprogesterone acetate (UV 240), medroxyprogesterone (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone acetate (UV 240), methylprednisolone (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisolone acetate (UV 240), prednisolone (UV 240), prednisone (UV 240), pregnenolone (UV 210), progesterone (UV 240), testosterone (UV 240)

REFERENCE
Sadlej-Sosnowska,N. Structure retention relationship for steroid hormones. Functional groups as structural descriptors, *J.Liq.Chromatogr.*, **1994**, *17*, 2319–2330.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 1 μM solution in MeOH.

HPLC VARIABLES
Column: 470 × 4.6 5 μm Spheri-5 RP-18
Mobile phase: MeOH:water 56:44
Flow rate: 0.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Simultaneous: dehydrocorticosterone, hydrocortisone, methylprednisolone, prednisolone, prednisone, tetrahydrocortisol, tetrahydrocortisone

REFERENCE
Lukulay,P.H.; McGuffin,V.L. Comparison of solvent modulation with premixed mobile phases for the separation of corticosteroids by liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 4039–4062.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 10 μL aliquot of a 100 ppm solution.

HPLC VARIABLES
Column: 150 × 4.6 Develosil ODS-5
Mobile phase: Gradient. MeOH:water from 50:50 to 90:10 over 15 min
Flow rate: 1
Injection volume: 10
Detector: MS, JEOL JMS-SX102A reversed geometry (BE), accelerating voltage +5 kV, air pressure chemical ionization APCI, nebulizer 290°, ion source chamber 400°, discharge electrode, skimmer 1 aperture 300 μm, skimmer 2 aperture 400 μm, no nebulizer gas

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: corticosterone, hydrocortisone, progesterone

REFERENCE
Nojima,K.; Fujimaki,S.; Hertsens,R.C.; Morita,T. Application of liquid chromatography-atmospheric pressure chemical ionization mass spectrometry to a sector mass spectrometer, *J.Chromatogr.A*, **1995**, *712*, 17–19.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in n-propanol:water 80:20 or DMF:water 80:20, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: Gradient. A was hexane. B was ethyl acetate. C was 0.1% formic acid in MeCN. D was 0.1% formic acid in water. A:B:C:D 100:0:0:0 for 5 min, to 0:100:0:0 over 15 min, maintain at 0:100:0:0 for 5 min, to 0:0:100:0 over 5 min, maintain at 0:0:100:0 for 5 min; to 0:0:0:100 over 25 min, maintain at 0:0:0:100 for 5 min.
Flow rate: 0.9
Detector: Evaporative light scattering (Sédex 55, Sédéré)

CHROMATOGRAM
Retention time: 21.90

OTHER SUBSTANCES
Simultaneous: acetylcholine, cholesterol, choline, dextrose, estradiol, glycine, phenylalanine, sodium, testosterone

REFERENCE
Treiber,L.R. Normal-phase high-performance liquid chromatography with relay gradient elution. I. Description of the method, *J.Chromatogr.A*, **1995**, *696*, 193–199.

SAMPLE
Matrix: urine
Sample preparation: Condition a 10 mL 200 mg MCF Isolute SPE cartridge with two 3 mL portions of EtOH and two 3 mL portions of water. Centrifuge urine at 4000 g for 30 in, filter through a 0.22 μm filter unit. Dilute 0.75-3mL urine to 4 mL with water. Add 30 ng IS. Add to the SPE cartridge. Wash with three 3 mL portions of water, 3 mL MeOH: 10 mM NaOH 30:70, twice with 3 mL water and with 3 mL MeOH:10 mM HCl 30:70. Elute with 3 mL EtOH. Evaporate eluate under vacuum and reconstitute the residue with 150 μL mobile phase. Inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 3.2 5 μm Nucleosil 120-C18
Mobile phase: MeCN:water 24:76
Flow rate: 0.5
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 11.45
Internal standard: dexamethasone (22.01)
Limit of detection: 3.8 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, hydrocortisone

KEY WORDS
SPE; human; pig

REFERENCE
Hay,M.; Mormède,P. Improved determination of urinary cortisol and cortisone, or corticosterone and 11-dehydrocorticosterone by high-performance liquid chromatography with ultraviolet absorbance detection, *J.Chromatogr.B*, **1997**, *702*, 33–39.

SAMPLE
Matrix: urine
Sample preparation: 3 mL Urine + 1.5 μg betamethasone + 100 mg K_2HPO_4 + 500 mg anhydrous sodium sulfate + 5 mL diethyl ether, shake mechanically for 10 min, centrifuge at 2500 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject a 15 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. MeCN:water from 4:96 to 30:70 over 10 min, to 45:55 over 5 min, to 50:50 over 3 min
Column temperature: 40
Flow rate: 1
Injection volume: 15
Detector: UV 246

CHROMATOGRAM
Retention time: 11.46
Internal standard: betamethasone (12.83)
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: corticosterone, deoxycorticosterone, hydrocortisone, 11α-hydroxyprogesterone, prednisolone, prednisone, triamcinolone, triamcinolone acetonide

REFERENCE
Park,S.-J.; Kim,Y.-J.; Pyo,H.-S.; Park,J. Analysis of corticosteroids in urine by HPLC and thermospray LC/MS, *J.Anal.Toxicol.*, **1990**, *14*, 102–108.

SAMPLE
Matrix: urine
Sample preparation: 3 mL Urine + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na_2HPO_4, add 4 mL dichloromethane, vortex 1 min, centrifuge at 3700 g for 3 min. Remove organic phase and dry it over anhydrous sodium sulfate. Evaporate a 3 mL aliquot to dryness under vacuum, reconstitute residue with 200 μL 5 μg/mL IS in MeOH, inject 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Hypersil ODS
Mobile phase: MeCN:water 32:68
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM
Retention time: 5.3

Internal standard: methylprednisolone (9)

OTHER SUBSTANCES

Simultaneous: triamcinolone, triamcinolone acetonide, prednisone, dexamethasone, betamethasone, corticosterone, hydroxyprogesterone, fluorocortisone acetate, hydrocortisone, fluorocortisone
Interfering: prednisolone

KEY WORDS

SPE also discussed

REFERENCE

Santos-Montes,A.; Gonzalo-Lumbreras,R.; Gasco-Lopez,A.I.; Izquierdo-Hornillos,R. Solvent and solid-phase extraction of natural and synthetic corticoids in human urine, *J.Chromatogr.B*, **1994**, *652*, 83–89.

SAMPLE

Matrix: urine
Sample preparation: 3 mL Urine + 100 ng methylprednisolone + 0.25 g NaCl, adjust pH to 9.0 with 0.5 g Na_2HPO_4, add 4 mL dichloromethane, vortex 1 min, centrifuge at 3700 g for 3 min. Remove organic phase and dry it over anhydrous sodium sulfate. Evaporate a 3 mL aliquot to dryness under vacuum, reconstitute residue with 200 μL MeOH, inject 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil 5-ODS
Mobile phase: MeCN:water 30:70
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 245

CHROMATOGRAM

Retention time: 6.5
Internal standard: methylprednisolone (14)
Limit of detection: 55 pg

OTHER SUBSTANCES

Extracted: hydrocortisone
Simultaneous: fluorocortisone
Noninterfering: corticosterone, deflazacort, deoxycorticosterone, fluorocortisone acetate, 21-hydroxydeflazacort, 11α-hydroxyprogesterone, prednisolone, prednisone, triamcinolone acetonide

REFERENCE

Santos-Montes,A.; Gonzalo-Lumbreras,R.; Izquierdo-Hornillos,R. Simultaneous determination of cortisol and cortisone in urine by reversed-phase high-performance liquid chromatography. Clinical and doping control applications, *J.Chromatogr.B*, **1995**, *673*, 27–33.

SAMPLE

Matrix: urine
Sample preparation: 10 mL Urine + 40 μL 25 μg/mL corticosterone, vortex briefly, add 1 mL 100 mM NaOH, vortex briefly, add 3 mL dichloromethane, rotate at 20 rpm for 45 min, centrifuge at 1000 g for 15 min, discard the aqueous layer, centrifuge at 1000 g for 10 min, discard the aqueous layer, add 150 mg NaCl, break up emulsion, centrifuge for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 45°, reconstitute the residue in 150 μL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. MeOH:water from 30:70 to 44:56 over 6 min, maintain at 44:56 for 14 min, return to initial conditions over 3 min, re-equilibrate for 5 min.
Flow rate: 1
Detector: UV 246

CHROMATOGRAM

Retention time: 12.8
Internal standard: corticosterone (17.8)

OTHER SUBSTANCES

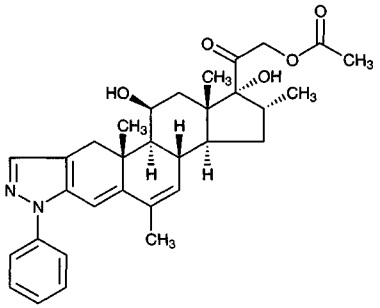
Extracted: hydrocortisone

REFERENCE

Lee,Y.S.; Lorenzo,B.J.; Koufis,T.; Reidenberg,M.M. Grapefruit juice and its flavonoids inhibit 11β-hydroxysteroid dehydrogenase, *Clin.Pharmacol.Ther.*, **1996**, *59*, 62–71.

Cortivazol

Molecular formula: $C_{32}H_{38}N_2O_5$
Molecular weight: 530.66
CAS Registry No.: 1110-740-3
Merck Index: 2603



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM

Retention time: 12.002

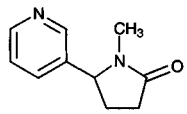
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Cotinine



Molecular formula: $C_{10}H_{12}N_2O$

Molecular weight: 176.22

CAS Registry No.: 486-56-6, 5695-98-7 (fumarate)

Merck Index: 2619

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 4.7

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: meconium

Sample preparation: Condition a 3 mL C8 SPE cartridge (Backer-Bond, Gross Gerau, Germany) with 5 mL MeOH and 5 mL water. Condition a 3 mL silica SPE cartridge (Backer-Bond) with 5 mL chloroform. Place the C8 SPE cartridge on top of the silica SPE cartridge. Thaw and mix meconium, weigh a 2 g aliquot, add IS, and emulsify in 20 mL 100 mM pH 8.0 phosphate buffer. Vortex, centrifuge, filter the supernatant. Extract the supernatant 3 times with 2 mL portions of chloroform. Evaporate chloroform extracts to dryness and dissolve in 10 mL pH 9.0 boric buffer. Add to the SPE cartridges, remove the C8 column and elute the silica column with 3 mL MeOH:dichloromethane:ammonium hydroxide 30:70:1. Evaporate the eluate to dryness under nitrogen and redissolved in 100 μL water. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm LiChrosorb C18
Mobile phase: MeCN:MeOH:water:pH 4.66 acetate buffer: acetic acid 2:29:50:20:1, pH adjusted to 4.3 with diethylamine
Flow rate: 1.0
Injection volume: 20
Detector: UV

CHROMATOGRAM

Retention time: 7.29
Internal standard: ephedrine
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Extracted: caffeine, nicotine

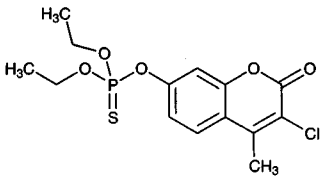
KEY WORDS

SPE

REFERENCE

Baranowski,J.; Pochopien,G.; Baranowska,I. Determination of nicotine, cotinine and caffeine in meconium using high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *707*, 317–321.

Coumaphos



Molecular formula: $C_{14}H_{16}ClO_5PS$
Molecular weight: 362.77
CAS Registry No.: 56-72-4
Merck Index: 2626

SAMPLE

Matrix: blood, tissue
Sample preparation: Homogenize mouse brain in ten volumes 100 mM pH 7.4 sodium phosphate buffer. 2 mL Plasma or homogenate + 1 g NaCl + 2 mL ethyl acetate, vortex for 30 s, centrifuge at 1000 g for 10 min, repeat extraction. Combine the organic layers and evaporate them under a stream of nitrogen.

HPLC VARIABLES

Column: 300 × 4 μPorasil
Mobile phase: Dichloromethane:glacial acetic acid 100:0.02
Flow rate: 1
Detector: UV 290

CHROMATOGRAM

Retention time: 10.0
Internal standard: coumaphos

OTHER SUBSTANCES

Extracted: parathion, chlorpyrifos

KEY WORDS

plasma; rat; mouse; microsomes; brain; coumaphos is IS

REFERENCE

Sultatos,L.G.; Costa,L.G.; Murphy,S.D. Determination of organophosphorus insecticides, their oxygen analogs and metabolites by high pressure liquid chromatography, *Chromatographia*, **1982**, *15*, 669–671.

SAMPLE

Matrix: eggs, milk
Sample preparation: Prepare a SPE column from 500 mg silanized Celite 545 followed by 5 g Nuchar S-N:silanized Celite 545 1:4 in a 22 mm i.d. chromatographic column, wash with 50 mL MeCN:toluene 75:25. Eggs. 150 g Chopped eggs + 300 mL MeCN, homogenize (Polytron) at half speed for 30 s and full speed for 1 min, vacuum filter (S & S 597 paper). Remove an aliquot equivalent to 100 g sample, make up to 100 mL with water, add 500 μL hexadecane to reduce foaming, concentrate to 75 mL on a rotary evaporator at 35°. Place mixture in a separatory funnel, add 15 g NaCl, shake until dissolved, wash the original flask with three 25 mL aliquots of MeCN, add the washings to the separatory funnel, shake 30 s, let separate for 5 min, remove the MeCN layer, extract the aqueous layer with 50 mL MeCN. Combine the MeCN layers, wash with 25 mL 20% NaCl, wash with 100 mL petroleum ether, add 50 mL 2% NaCl, extract with 100, 25, and 25 mL portions of dichloromethane. Combine lower organic layers and pass them through a 50 × 22 column of anhydrous sodium sulfate, evaporate eluate to dryness on a rotary evaporator at 35°, reconstitute in 10 mL dichloromethane, add to SPE column, wash in with 10 mL dichloromethane, wash in with 25 mL MeCN:toluene 75:25, elute with 100 mL MeCN:toluene 75:25 at 5 mL/min, evaporate eluate to dryness on a rotary evaporator at 35°, reconstitute in 5 mL MeOH, filter 5 μm, inject a 10 μL aliquot of the filtrate. Milk. 150 g Milk + 300 mL acetone, shake vigorously for 1 min, centrifuge at 1200 rpm for 5

min. Remove 277 mL supernatant and add it to 15 mL water, concentrate to 75 mL on a rotary evaporator at 35°. Place mixture in a separatory funnel, add 15 g NaCl, shake until dissolved, wash the original flask with three 25 mL aliquots of MeCN, add the washings to the separatory funnel, shake 30 s, let separate for 5 min, remove the MeCN layer, extract the aqueous layer with 50 mL MeCN. Combine the MeCN layers, wash with 25 mL 20% NaCl, wash with 100 mL petroleum ether, add 50 mL 2% NaCl, extract with 100, 25, and 25 mL portions of dichloromethane. Combine lower organic layers and pass them through a 50 × 22 column of anhydrous sodium sulfate, evaporate eluate to dryness on a rotary evaporator at 35°, reconstitute in 10 mL dichloromethane, add to SPE column, wash in with 10 mL dichloromethane, wash in with 25 mL MeCN:toluene 75:25, elute with 100 mL MeCN:toluene 75:25 at 5 mL/min, evaporate eluate to dryness on a rotary evaporator at 35°, reconstitute in 5 mL MeOH, filter 5 μm, inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 70 × 2.1 25-37 μm Co:Pell ODS
Column: 250 × 4.6 6 μm Zorbax C8
Mobile phase: Gradient. MeCN:water 12:88 to 70:30 over 30 min, then 100:0 for 5 min, re-equilibrate for 10 min.
Column temperature: 35
Flow rate: 1.5
Injection volume: 10
Detector: F ex 320 em 385

CHROMATOGRAM

Retention time: 31
Limit of detection: <10 ppb

KEY WORDS

SPE

REFERENCE

Krause,R.T.; Min,Z.; Shotkin,S.H. Determination of coumaphos and its oxygen analog in eggs and milk by using a multiresidue method with liquid chromatographic quantitation and capillary gas chromatographic/mass spectrometric confirmation, *J.Assoc.Off.Anal.Chem.*, **1983**, *66*, 1353–1357.

SAMPLE

Matrix: formulations
Sample preparation: Dilute formulation 100-fold with MeOH, centrifuge at 1250 g for 10 min, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 30 × 4.6 3 μm P-E 3 × 3 C18 (Perkin-Elmer)
Mobile phase: MeCN:water 85:15
Flow rate: 2
Injection volume: 10
Detector: UV 313

CHROMATOGRAM

Retention time: 0.3
Limit of detection: 300 pg

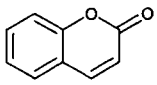
OTHER SUBSTANCES

Also analyzed: amitraz (UV 313), chlorpyrifos (UV 313), crotoxyphos (UV 229), permethrin (UV 229), phosmet (UV 229)

REFERENCE

Rice,L.G. Rapid separation of pesticides by high-performance liquid chromatography with 3-μm columns, *J.Chromatogr.*, **1984**, *317*, 523–526.

Coumarin



Molecular formula: $C_9H_6O_2$
Molecular weight: 146.15
CAS Registry No.: 91-64-5
Merck Index: 2630

SAMPLE

Matrix: blood, tissue
Sample preparation: Condition a silica Sep-Pak SPE cartridge also containing 2 g sodium sulfate (?) with 5 mL MeOH and 5 mL cyclohexane. Mix 3 mL blood or crushed tissue with 1 mL 20 μg/mL IS, adjust to pH 3-4 with 0.5 M sulfuric acid, extract three times with 10 mL MeOH:chloroform 10:90 (Caution! Chloroform is a carcinogen!). Evaporate at 40°, re-dissolve the residue in 5 mL cyclohexane, sonicate and centrifuge three times. Remove a 5 mL aliquot of the top layer, evaporate at 40°. Reconstitute the residue in 5 mL cyclohexane. Add to the SPE cartridge, elute with 5 mL MeOH, evaporate at 40°, reconstitute the residue in MeOH, inject an aliquot.

HPLC VARIABLES

Column: 200 mm long μBondapak C18
Mobile phase: MeOH:0.8% acetic acid 80:20
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM

Retention time: 3.9
Internal standard: N,N-diphenylbenzidine (9.3)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES

Extracted: bromadiolone, brodifacoum, coumatetralyl, warfarin

KEY WORDS

SPE; plasma; heart; lung; liver; kidney; spleen

REFERENCE

Park,S.W.; Seo,B.S.; Kim,E.H.; Kim,D.H.; Paeng,K.-J. Purification and determination procedure of coumarin derivatives, *J.Forensic Sci.*, **1996**, *41*, 685–688.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.